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THE ELECTRICAL ACTIVITY OF THE BUNDLE OF HIS

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The initial purpose of the present study was to find an explanation for the long time interval elapsing between the activation of the auricle and the ventricle. Considering the fact that the auriculo-ventricular (A.V.) node is a structure which the impulse coming from the auricle traverses on its way towards the ventricle, it seemed likely that an explanation for the A.V. latency could be found by studying its activity. It was also expected that the node should reveal its activity through an action potential which in turn could be registered by appropriate means.

Aside from the histological description of the bundle of His given by Kent (1893) and His (1893) very little is known about the functional properties this tissue possesses. Our present knowledge regarding the physiological properties of the bundle of His can very well be summarized by the conclusions reached by Kent (1893) in his classical study. Kent demonstrated that the propagation of impulse from the auricle to the ventricle is accomplished '. . . by strands of altered muscular tissue. . .', this tissue later appearing in the literature under the name of the bundle of His.

In the course of the present study sufficient evidence was gathered to conclude that the action potential recorded did not legitimately belong to the A.V. node, but was due to the activity of the bundle of His. An analysis of this action potential was carried out and some of its physiological properties were studied.

METHODS

The experiments were carried out on isolated perfused hearts of dogs and cats. The cats' hearts were perfused according to Langendorff's procedure. The perfusion of the dog's hearts was done through the aorta with blood from another dog which served as a donor. The donor dog was anaesthetized with sodium pentobarbitone (Nembutal, Abbott Laboratories, Ltd., 33 mg/kg body wt., I.V.), and heparinized. The technique employed was the modification introduced by García-Ramos, Alanís & Rosenblueth (1950) to the method of Heymans & Kochmann (1904). The electrical

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activity from the interauricular or interventricular septum was recorded through a T-shaped incision on the right auricular wall. The edges of the cut wall were everted by inserting small stainless-steel hooks. The heart was suspended over a glass funnel which was used to collect the overflowing blood and to attach the hooks on its upper edge. The preparation is shown diagrammatically in Fig. 1. This technique offers a good exposure of the A.V. groove, the interauricular septum and the upper part of the interventricular septum, as well as the orifice of the coronary sinus. The anterior edge of the coronary sinus was taken as the main reference to locate the zone, where the exploring electrodes should be introduced. The insertion of the A.V. valves was severed to facilitate the positioning of the electrodes.

In the majority of the experiments the heart was stimulated with rectangular pulses, applied through a pair of small stainless-steel clamps placed on the upper third of the endocardial surface of the auricle. The pulses were provided by a Grass stimulator (Model S 4 A), passed through an isolating unit in order to eliminate the connexion with ground. Usually the stimulation intensity was betwen 3 and 5 times the threshold.

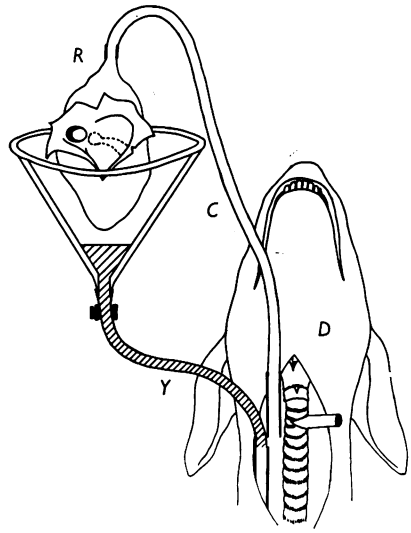

Fig. 1. Diagrammatic representation of the preparation used to perfuse the isolated heart of the dog. *D*, donor dog; *R*, perfused heart; *C*, connecting tube between the carotid artery of the donor and the aorta of the perfused heart; *Y*, connecting tube between the collecting funnel and the jugular vein of the donor. The auricle has been opened. Note the orifice of the coronary sinus which helps to locate the A.V. node and zone of bundle of His (broken line).

In some experiments the heart was beating spontaneously, while in others the sino-auricular (S.A.) node was crushed so that the A.V. node became the pace-maker. In order to block the conduction within the bundle of His it was severed in the upper right angle of the interventricular septum. The recording electrodes used were steel needles 4–5 mm long, with their tips sharpened to a diameter of 20–50 μ. The other end of the needle was soldered to a thin wire coil (0·1 mm diameter). The needles were covered with an isolating material (Polystyrene) leaving only the thin tips uncovered. This type of electrodes, which will be referred to as exploring electrodes, proved to be very advantageous when the heart was beating, because they easily follow the movement of the heart and so reduce the mechanical artifacts to a minimum.

The potentials were recorded with a double-beam cathode-ray oscilloscope, previously amplified by a 3-stage push-pull capacity resistance-coupled amplifier.

In some experiments the vagus nerves were stimulated. In this case they were left attached to the isolated heart and stimulated through Sherrington type electrodes. The stimulation frequency varied from 5 to 20 c/s. The substances to be studied were injected directly into the aortic tube of the receptor heart.

RESULTS

When the exploring electrodes were introduced into the region where the A.V. node and the bundle of His are known to be located a small action potential was recorded. The temporal relationships and the characteristics of this potential (H potential, Fig. 2) suggested that it neither belonged to the

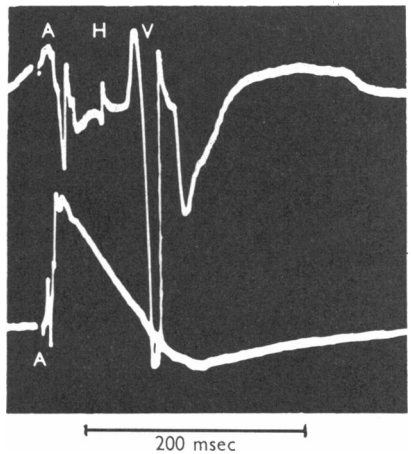

Fig. 2. Action potential recorded from the zone of bundle of His. The upper tracing shows the electrogram recorded by the exploring electrode: A, auricular electrogram; H potential from the bundle of His; V, ventricular electrogram. The lower tracing shows the auricular monophasic electrogram. The H potential appears when the slow component of the auricular electrogram has not yet concluded. The heart was stimulated at a constant frequency. Time calibration, 200 msec.

auricular nor to the ventricular electrogram. The experimental findings were similar in the hearts of cats and dogs. The description will be limited to the results obtained with the dog heart preparation, since it proved to be the more stable one.

Zone where the H potential could be registered

This zone was small, as can be seen in Figs. 3 and 4. In the experiment illustrated in Fig. 3, the potential was recorded with one electrode introduced into the region of the bundle of His and the other one placed on the periaortic connective tissue, this in turn being led to earth. Another pair of electrodes was used, one placed above and the other below the exploring electrode. The latter pair did not register the H potential; it only showed the auricular and ventricular electrograms.

Fig. 4 shows the H potential on both tracings, but it did not appear simultaneously, the lower one preceding the upper one by several milliseconds. Recordings taken at various points along the bundle of His show that the greater the distance from the coronary sinus the later the occurrence of the H potential.

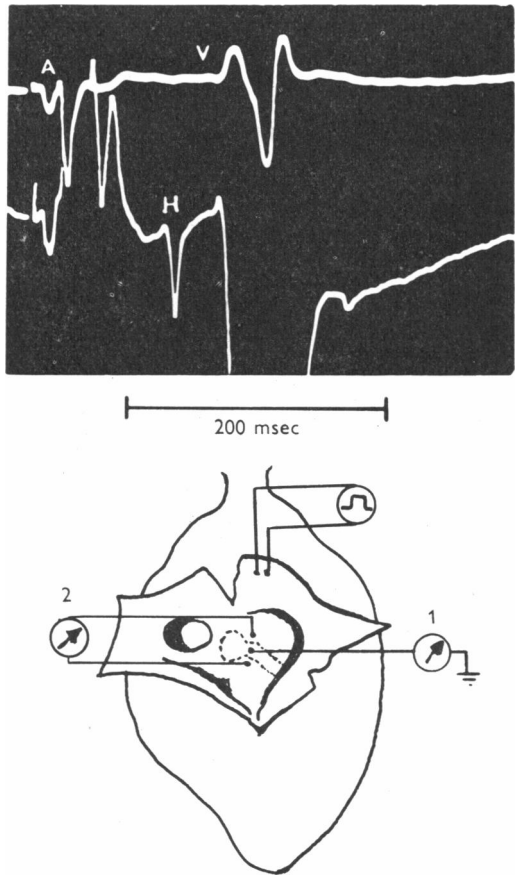

Fig. 3. Zone where the H potential can be registered. The diagram shows the position of the stimulating and recording electrodes. The lower tracing reproduces the record from the exploring electrode (1); the upper tracing the record when the electrodes (2) were placed 1·5 mm above and below the exploring electrode. The H potential was only seen in the records when the electrode was in the zone of bundle of His. Time calibration: 200 msec.

When the exploring electrode was introduced into the interauricular septum, 6 or 7 mm in front of the coronary sinus orifice and 1 mm above the A.V. groove, the H potential was registered (Fig. 4, lower tracing). Another exploring electrode placed in front of the first one revealed a 2 mm broad surface which extended down to the anterosuperior angle of the interventricular

septum, where the H potential could also be registered. The depth to which the exploring electrodes were introduced varied from 1 to 2 mm.

This localization coincides with that given in the anatomical descriptions of the bundle of His (Testut, 1924; Lev, Widdraw & Erickson, 1951). The farther the exploring electrode was introduced from the orifice of the coronary sinus, the longer the interval between the beginning of the auricular electrogram and the H potential (Fig. 4).

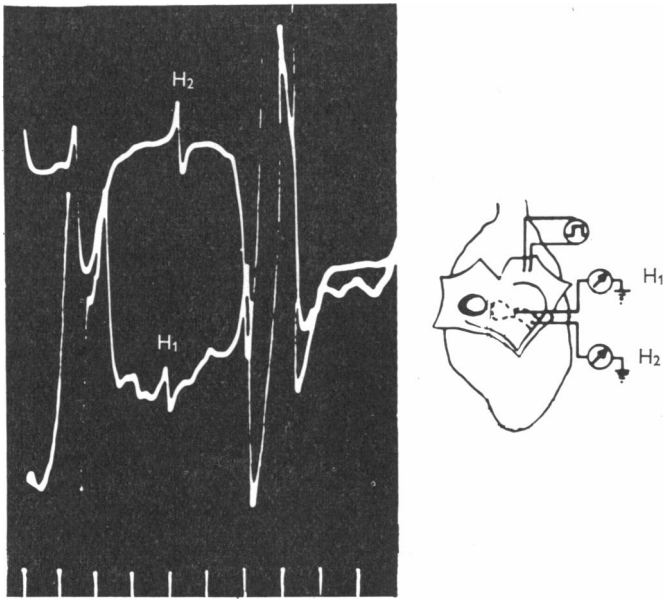

Fig. 4. Time differences in the records taken from two different points in the bundle of His. Dog heart auricle stimulated at constant frequency. The diagram shows the position of the stimulating and recording electrodes. The H_1 potential (lower tracing) was taken from the electrode H_1 nearest to the anterior edge of the coronary sinus; the H_2 potential (upper tracing) corresponds to the activation of the place situated beneath the H_2 electrode. Time marker, 20 msec. In any part of the region located between these two electrodes it was possible to register the H potential. Note the difference between the activation times of H_1 and H_2 and the similarity of their shape and amplitude.

The shape and amplitude of this potential were similar in all the tracings where it could be detected, and were independent of the site from which they were obtained. The H potential may be described as a diphasic wave (Fig. 5); its amplitude varied from 0·8 to 2·0 mV.

Relationships of the H potential with the auricular and ventricular electrograms

The H potential appeared between the auricular and the ventricular electrograms. To make the description easier, the auriculo-ventricular interval (A-V) may be subdivided into two subintervals, namely; the A-H and H-V

subintervals (Fig. 2). For the A-H subinterval the mean value was 70·5 msec with extremes between 148·0 and 36·0; for the H-V subinterval the mean value was 32·0 msec with extremes from 59·0 to 24·0. If the A-V interval is taken as 100%, the average percentages belonging to the A-H and the H-V subintervals were 60·3 and 39·7 msec respectively.

To prove that the H potential is an independent phenomenon, belonging neither to the auricular nor to the ventricular electrograms, the following observations were carried out. The S.A. node was destroyed to allow the heart to be activated by impulses from the A.V. node. In this case (Fig. 6) the first

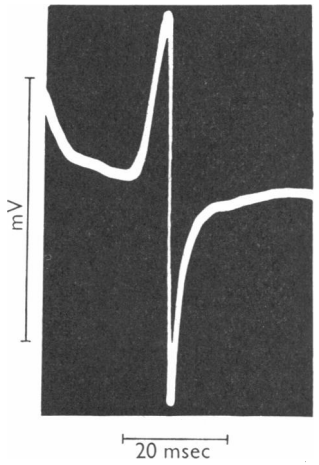

Fig. 5. Shape of the H potential. The tracing was taken with a pair of electrodes, having a tip of 30 μ diameter; separation between the electrodes, 0·7 mm. The pair was introduced into the bundle of His. Calibrations, 1·0 mV. and 20 msec.

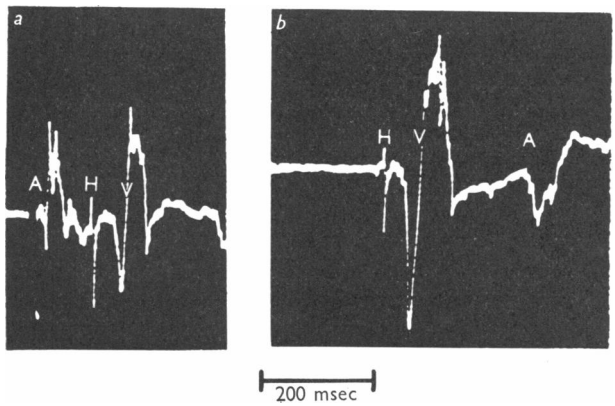

Fig. 6. Independence of the H potential with respect to the auricular electrogram. Dog heart (*a*) before and (*b*) after destruction of the S.A. node. In *a* the auricle was stimulated at constant frequency. In *b* the S.A. node had been destroyed and the heart followed the automatism of the A.V. node with the sequence H-V-A. The H-V interval remained approximately constant. Time calibration: 200 msec.

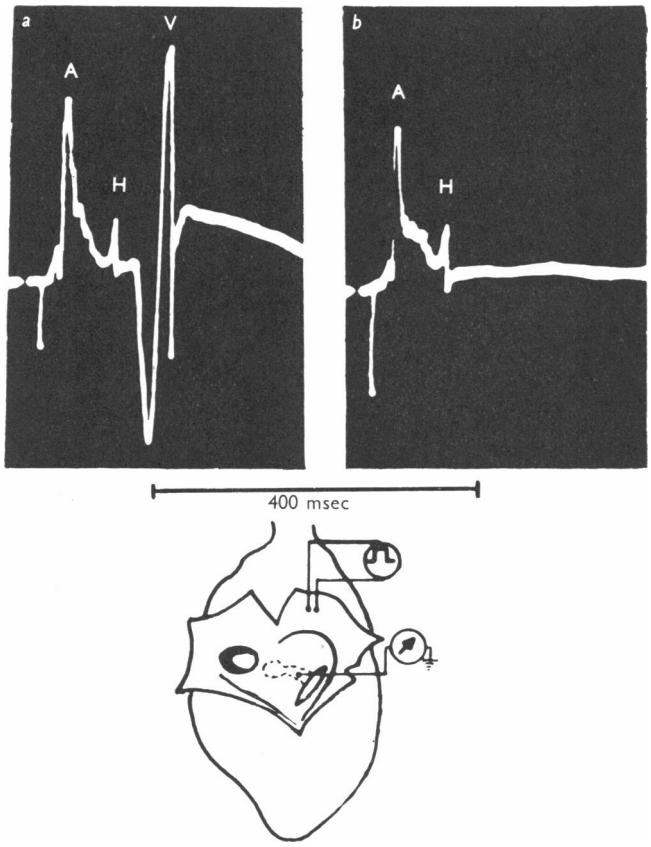

Fig. 7. Presistence of the H potential after severance of the bundle of His. The drawing illustrates the placing of the recording and stimulating electrodes as well as the site where the bundle was severed. Records *a* taken before the severance. After the severance (*b*), the H potential persists without being followed by the ventricular electrogram. Time calibration: 400 msec.

potential registered was the H potential; it was followed by the ventricular electrogram with its usual temporal relationships. The auricle was activated somewhat later. In other experiments the bundle was severed at the antero-superior angle of the interventricular septum; the tracings obtained (Fig. 7) show that the H potential was not followed by a ventricular electrogram.

Factors which modify the A-H subinterval

When the auricle was stimulated with increasing frequencies, the A-H subinterval became gradually longer. With high frequencies the A-V transmission failed altogether; the H potential and the ventricular electrogram disappeared simultaneously. The lengthening of the A-H subinterval is illustrated in Fig. 8. The average of the maximal increases of the A-H subinterval,

expressed as a percentage of the initial value, i.e. the one obtained with low frequencies, was 142·7 with extreme values between 279·0 and 44·0. The H-V subinterval was practically unaffected when the frequency of stimulation was raised. The average maximal frequency which the H potential and the ventricular electrogram could follow, when the auricle was stimulated, was 4·52/sec.

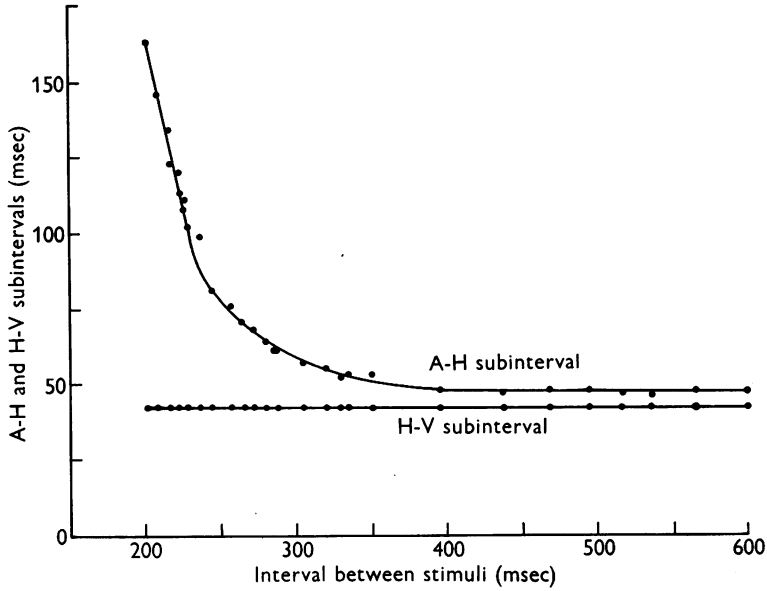

Fig. 8. Variations of the A-H subinterval (upper curve) and the H-V subinterval (lower curve) produced by the increase of the frequency of auricular stimulation. Abscissae, time interval between stimuli (msec). Ordinates, A-H and H-V subintervals (msec). The last dot of the curve coincides with the maximal frequency the ventricle was able to follow; the H potential also disappeared.

Mechanical block of A-V transmission

It was possible to produce an A.V. block by introducing an exploring electrode into a 2 mm wide region which corresponded to the anatomical localization of the A.V. node. This block was frequently reversible when the exploring electrodes were removed. The block was evidenced by the simultaneous disappearance of the H potential and of the ventricular electrogram.

Stimulation of the vagus and the effect of acetylcholine

The stimulation of the vagus nerve produced a lengthening of the A-H subinterval. This lengthening could be observed even if the frequency of stimulation of the auricle was kept constant. The degree of lengthening varied with the frequency of vagus stimulation; with high frequencies considerable lengthenings appeared and the impulses could even be blocked, above the

bundle of His, so that the H potential and the ventricular electrogram both disappeared (Fig. 9). When acetylcholine was infused at different concentrations, which varied from 50 to 400 μg/min, the results were similar to those obtained with vagus stimulation (Fig. 9). Neither vagus stimulation nor acetylcholine injections produced appreciable changes in the H-V subinterval.

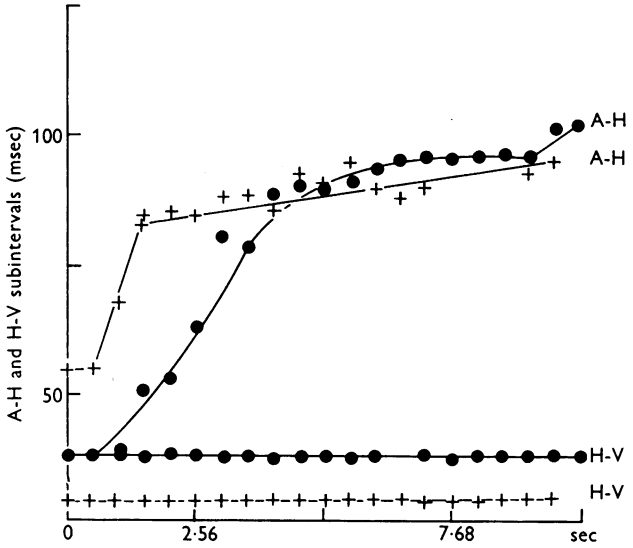

Fig. 9. Changes of the A-H and H-V subintervals produced by vagal stimulation (+, 20/sec) and by injections with acetylcholine 40 μg/min (●). In both cases the auricular frequency of stimulation was kept constant. Abscissae, time (sec); the origin represents the beginning of the electrical stimulation of the vagus and of the injection of acetylcholine; each symbol on the curves represents a cardiac cycle. Ordinates, A-H and H-V values (msec).

Asphyxia and the modifications of the A-H and H-V subintervals

When the heart was subjected to periods of asphyxia by the occlusion of the aortic cannula, changes in the A-H subinterval were observed which resembled those seen when the auricular frequency of stimulation was increased, or when the vagus was stimulated, or acetylcholine was injected; that is, the A-H subinterval was lengthened up to the point where the H potential and the ventricular electrogram disappeared. The duration of these periods of asphyxia varied from 3 to 5 min; the changes were reversible. The duration of the H-V subinterval does not vary appreciably during asphyxia.

The effects of adrenaline

The infusion of adrenaline (Adrenaline, Colliere), at concentrations of 4–30 μg/min into the heart, shortened both the A-V and H-V subintervals (Fig. 10*A*). This was not due to any changes in heart rate since the frequency of stimulation was high enough to avoid these changes. The A-H subinterval

was shortened by an average of 36·0% and the H-V by 13·0% as compared with the control.

An increase in the frequency of auricular stimulation during constant infusion of adrenaline produced a lengthening of the A-H subinterval, while the H-V subinterval shortened further. The limiting frequency which the H potential and the ventricular electrogram followed was higher than that followed when the frequency was increased before adrenaline had been infused (Fig. 10*B*).

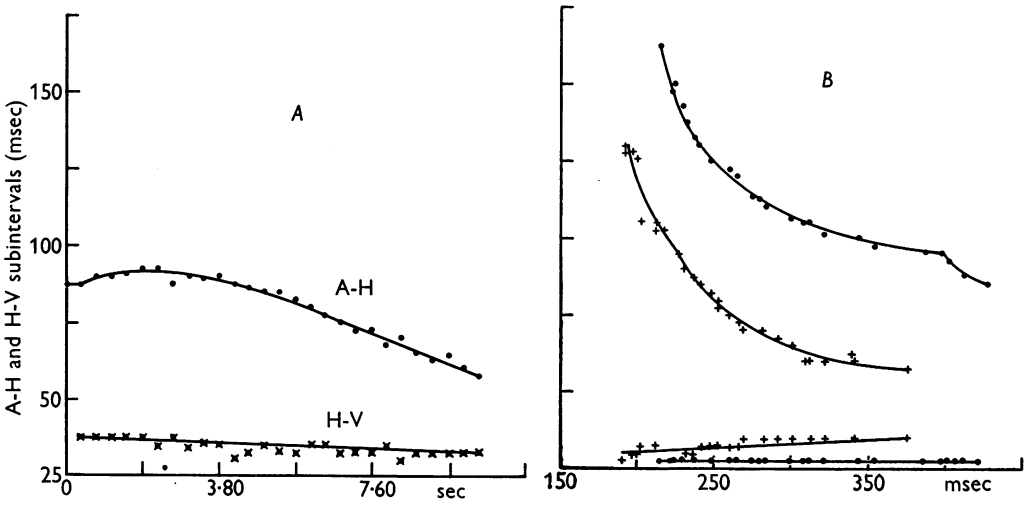

Fig. 10. *A*: effects of adrenaline on the A-H, ●, and H-V, ×, subintervals. Abscissae, time (sec). Adrenaline, 10 μg/min, began to be injected at time zero. Each point corresponds to a cardiac cycle. Heart stimulated at constant frequency. *B*: increase of the frequency of auricular stimulation during a constant injection of adrenaline. Abscissae, intervals between auricular stimuli (msec). Dots, ●, control before adrenaline was injected; +, during the continuous injection of adrenaline.

DISCUSSION

The zone where the H potential can be registered is well delimited and its location coincides with the one to be expected in accordance with the anatomical description of the bundle of His by Kent (1893) and by His (1893). When the exploring electrodes are introduced above or below this zone or very near to the anterior edge of the coronary sinus orifice it is not possible to register the H potential. When the electrode is introduced into different places, but following the course of the bundle, the H potential is seen in all tracings. This evidence justifies the hypothesis that the H potential originates in the bundle of His. It might be thought that this potential originates in the A.V. node and is propagated electrotonically in view of the fact that the A.V. node is located in the same area where the H potential is

recorded. However, the following findings tend to rule out this hypothesis. The amplitude of the H potential does not change along the course of the bundle of His, whereas in the case of the H potential originating in the node a progressive reduction in amplitude would be expected when the leads are placed at greater distances from the A.V. node. The H potentials derived from different points are not synchronous; a progressive delay occurs along the bundle of His.

The diphasic responses obtained in the tracings depend on the recording method. With a discrete exploring electrode and another diffuse lead, the large conducting volume of the heart and circulating blood, monophasic tracings cannot be obtained. This technical limitation does not permit a measurement of the duration of the H potential.

Temporal relationships

To explain the H potential interposed between the auricular and ventricular electrograms, the following possibilities can be suggested: (*a*) that it belongs to the auricular electrogram; (*b*) that it belongs to the ventricular electrogram; (*c*) that it is an independent phenomenon produced by the previous activation of the auricle; or else (*d*) that it follows the activation of the A.V. node. The temporal relationships existing between the auricular electrogram and the production of the H potential are compatible with the first hypothesis. When the pace-maker was displaced towards the A.V. node, however, it was found that the H potential appeared without being preceded by an auricular electrogram. This fact eliminates the possibilities (*a*) and (*c*). The suggestion that the H potential is a part of the ventricular electrogram can also be eliminated, since the severance of the bundle of His causes the disappearance of the ventricular electrogram but the H potential persists (Fig. 7).

It may be concluded that the H electrogram represents the activation of the bundle of His, and does not require the previous activation of the auricle. It belongs neither to the auricular nor to the ventricular electrograms. This conclusion justifies the subdivision of the A-V interval into the subintervals A-H and H-V. Since the introduction of an exploring electrode into the region where the bundle of His is located does not produce block, while the introduction into the region of the A.V. node may do so, the activation of the bundle of His may be attributed to the activity previously originated in the A.V. node.

The influence of the frequency of stimulation on the A-H subinterval

The lengthening of the A-H subinterval during the increase of the frequency of stimulation can be explained in several ways. Since the conduction velocity of some cardiac tissues is diminished during the relative refractory period (Lewis, 1925) this lengthening could be due to a diminution of the conduction velocity of the bundle of His. The measurements of this velocity (J. Alanís &

E. López, unpublished observations) show that the changes are small and could thus explain only a small fraction of the lengthening of the A-H subinterval.

Another possible explanation of the lengthening of the A-H subinterval would be to assume the presence of some process or structure between the auricle and the ventricle, which through its functional characteristics could impose limitations on the auriculo-ventricular propagation. As can be seen in Fig. 8, the H-V subinterval is not appreciably modified when the frequency is increased. It may be concluded, therefore, that the lengthening of the A-V interval is mainly produced by the A-H subinterval. The lengthening of the A-V interval takes place in a region located above that where the H potential develops, i.e. above the bundle of His. This region corresponds to the A.V. node. The duration of the H-V subinterval is determined by the time of propagation of impulses through the His-Purkinje-ventricle system. Its constant duration is explained by the fact that its conduction velocity is not appreciably modified by the stimulation frequencies employed.

The simultaneous disappearance of the H potential and the ventricular electrogram when the frequency of auricular stimulation is sufficiently increased suggests that the frequency which the ventricle can follow, when the impulses come from the auricle, is limited, probably by the refractory period of the A.V. node. The activation of the ventricle depends on the presence of the H potential, i.e. on the previous activation of the bundle of His.

Other factors which lengthen the A-H subinterval

The lengthening of the A-H subinterval is not due exclusively to an increased heart rate. The stimulation of the vagus, injections of acetylcholine, and asphyxia produced the same effect on hearts in which the frequency of stimulation was kept constant (Fig. 9). Following the same reasoning as above it can be concluded that these factors produce their effects above the bundle of His, that is, in the region of the A.V. node.

The action of adrenaline

The shortenings of both the A-H and H-V subintervals produced by adrenaline are also independent of the modifications in frequency in heart rate since they appear when the heart is stimulated at a constant frequency (Fig. 10 *A*, *B*). Since adrenaline increases the conduction velocity of some cardiac tissues (Rosenblueth & García-Ramos, 1947; García-Ramos, Méndez & Rosenblueth, 1948; Krayer, Mandoki & Méndez, 1951), the shortening of the A-V and H-V subintervals may be due to this mechanism. The measurements of the conduction velocity of the bundle of His (J. Alanís and E. López, unpublished observations) show that adrenaline increases its conduction velocity, but this

increase explains only a small part of the shortening of the A-H subinterval. From this it may be concluded that the site at which adrenaline acts is again situated above the bundle of His, in the A.V. node.

SUMMARY

1. Hearts from cats and dogs were isolated and perfused. The right auricle was incised to explore the bundle of His and the A.V. node.

2. A special type of electrode is described which was used to avoid movement artifacts.

3. When the electrodes were placed in the region of the bundle of His an action potential was registered (H potential) intermediate between the auricular and ventricular electrograms. The potential was not obtained when the electrodes were placed 1 or 2 mm above or below this zone.

4. The H potential is independent of the auricular and ventricular electrograms since it may not be preceded by the auricular electrogram, and persists after the severing the bundle of His below the exploring electrode, and is then not followed by the corresponding ventricular electrogram. When the exploring electrodes were introduced in any place along the bundle of His, its shape and amplitude were similar.

5. Recordings taken at various points along the bundle of His show that the greater the distance from the coronary sinus the later the occurrence of the H potential.

6. The H potential subdivides the A-V interval into two subintervals: A-H and H-V.

7. The normal activation of the ventricle requires previous activity of the bundle of His, as is evidenced by the presence of the H potential.

8. When the auricle was stimulated with increasing frequencies, the A-H subinterval lengthened and the H-V subinterval remained constant. With high frequencies both the H potential and the ventricular electrogram disappeared simultaneously. Vagal stimulation, acetylcholine injections and asphyxia had similar effects.

9. Adrenaline shortened the A-H and H-V subintervals.

10. The mechanisms responsible for the lengthening and shortening of the A-H subinterval and for the simultaneous disappearance of the H and ventricular electrograms are discussed. It is concluded that these changes occur above the bundle of His, in the A.V. node.

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